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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/00, 5/00, 15/63, C12P 21/06, C12Q 1/68, A01K 67/00

A1

(11) International Publication Number: WO 00/55310

(43) International Publication Date: 21 September 2000 (21.09.00)

(21) International Application Number: PCT/US00/06862

(22) International Filing Date: 15 March 2000 (15.03.00)

(30) Priority Data:
09/268,303 15 March 1999 (15.03.99) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 09/268,303 (CIP)
Filed on 15 March 1999 (15.03.99)

(71) Applicant *(for all designated States except US)*: THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK [US/US]; West 116th Street and Broadway, New York, NY 10027 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: FISHER, Paul, B. [US/US]; 15 Gordon Place, Scarsdale, NY 10583 (US). GOPALKRISHNAN, Rahul [IN/US]; 302 West 79th Street, Apartment 2A, New York, NY 10024 (US).

(74) Agent: WHITE, John, P.; Cooper & Dunahm LLP, 1185 Avenue of the Americas, New York, NY 10036 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: IMPROVED EXPRESSION VECTOR FOR CONSISTENT CELLULAR EXPRESSION OF THE TET ON REPRESSOR IN MULTIPLE CELL TYPES

(57) Abstract

The present invention provides a vector comprising Protein Translation Peptide Elongation Factor–1 α promoter and nucleic acids encoding reverse tetracycline controlled transactivator, wherein the expression of said transactivator is under the control of Protein Translation Peptide Elongation Factor–1 α promoter. In addition, the invention provides a method of generating a reverse tetracycline controlled transactivator expression system for inducible tetracycline regulated gene expression comprising: (a) isolation of a DNA fragment encoding the reverse tetracycline controlled transactivator by restriction enzyme digestion, (b) generation of Protein Translation Peptide Elongation Factor–1 α promoter vector, by restriction enzyme digestion, (c) directional cloning of reverse tetracycline controlled transactivator into Protein Translation Peptide Elongation Factor–1 α promoter vector by ligation of 5' EcoRi compatible restriction enzyme overhangs, (d) directional cloning of reverse tetracycline controlled transactivator into Protein Translation Peptide Elongation Factor–1 α promoter vector by Klenow fragment mediated blunt end generation of 3' Bam HI end of DNA fragment encoding the reverse tetracycline controlled transactivator and 3' XbaI end of Protein Translation Peptide Elongation Factor–1 α promoter vector and (e) blunt cloning of partially ligated fragment to produce Protein Translation Peptide Elongation Factor–1 α promoter vector expressing reverse tetracycline controlled transactivator.